Structure-Based Design and Synthesis of Regioisomeric Disubstituted Aminoanthraquinone Derivatives as Potential Anticancer Agents

by **Hsu-Shan Huang***[a]), **Hui-Fen Chiu**[b]), **Pen-Fong Yeh**[c]), and **Chun-Lung Yuan**[a])

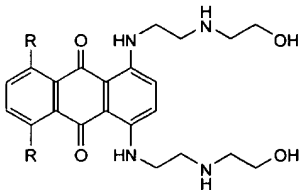
[a]) School of Pharmacy, National Defense Medical Center, Taipei, Taiwan, Republic of China (phone: +886-2-8792 64 54; fax: +886-2-8792 31 69; e-mail: huanghs@ndmctsgh.edu.tw)
[b]) Department of Pharmacology, Kaohsiung Medical University, Kaohsiung, R.O.C.
[c]) Cheng-Hsin Medical Center, Taipei, Taiwan, R.O.C.

Continuing our ongoing studies on cytotoxic substances, we report the synthesis and cytotoxic properties of a series of symmetric 1,5-diamino-9,10-anthraquinones with potentially bioreducible groups. Symmetric amination of 1,5-dichloro-9,10-anthraquinone with the appropriate primary amines in the presence of DMF furnished the structurally related aminoanthraquinone analogs **1**–**19**. Their *in vitro* cytotoxic activity was evaluated using rat glioma C6 cells, human hepatoma G2 cells, and 2.2.15 cells. Several compounds exhibited very high antitumor activities in these assays. Compound **4** efficiently inhibited C6 cells, human hepatoma G2 cells, and 2.2.15 cells, as determined by means of the XTT colorimetric assay. The antiproliferative activity of **4** was markedly enhanced, reaching a potency comparable to those of the powerful anticancer agents mitoxantrone, adriamycin, and cisplatin. Biological evaluations and structure/activity relationships within this class of novel aminoanthraquinones are discussed.

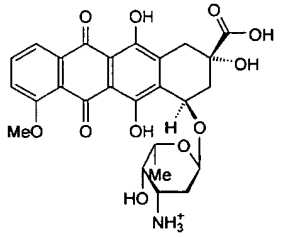
Introduction. – In recent years, intermolecular triple helices have found considerable interest as potential antigene agents that are able to inhibit the expression of particular genes in a sequence-specific fashion [1]. Several strategies have been proposed to increase the strength of such interactions [2], including *1*) covalent attachment of intercalating groups either at the ends [3–6] or within the third strand [7][8], *2*) covalent attachment of a positively charged moiety [9][10], or *3*) modification of the phosphodiester backbone [11][12].

As part of the work in our laboratory on the use of anthraquinone derivatives as anticancer agents or human telomerase activity agents, a number of doubly substituted 9,10-anthraquinones have been synthesized and biologically evaluated [13–16]. However, the mode of antitumor action of anthraquinone derivatives (*e.g.*, mitoxantrone) has not been clearly established yet. The possible modes include, among others, intercalation and binding to DNA, bioreduction, and aerobic redox cycling [17]. The 9,10-anthraquinone skeleton is a central constituent of the anthracyclines doxorubicin and daunorubicin, and of the anthracycline derivatives mitoxantrone and anthrapyrazole [18]. 9,10-Anthraquinone-based compounds currently occupy a prominent position in cancer chemotherapy, with the naturally occurring aminoglycoside-type anthracycline *doxorubicin* and the aminoanthraquinone *mitoxantrone* both in clinical use [19]. Mitoxantrone is an important synthetic aminoanthraquinone used in clinical management of leukemia and lymphoma, as well as in combination therapy for advanced breast and ovarian cancer [20]. Molecular modelling has been carried out for a number of amine-functionalized anthraquinone derivatives to determine their extent

of binding to G-tetraplex DNA, and their ability to inhibit the enzymes telomerase and Taq polymerase [21]. The planarity of the anthraquinone system allows intercalation into DNA, and its redox properties are linked to the production of radical species in biological systems [22]. Drug structures have been based upon the DNA-binding anticancer topoisomerase-II poison mitoxantrone with modified alkylamino side chains [23].



Mitoxantrone R = OH
Ametantrone R = H



Adriamycin

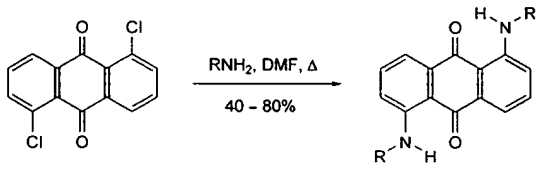
The anthracycline antibiotic adriamycin is a broad-spectrum antineoplastic agent with activity against a variety of tumors. Many adriamycin analogs, consisting of the anthraquinone skeleton with amino, alkylamino, and aminoalkylamino side chains, have been synthesized [24]. Diaminoanthraquinones were discovered initially as protein-kinase-C inhibitors, with IC_{50} values in the range of 50–100 μM with respect to *in vitro* tumor-cell growth, without cross-resistance to adriamycin [25]. *Adamson* proposed the removal of the aminosugar moiety from daunomycin and adriamycin and its replacement with an appropriate amino group to enhance antitumor activity [26].

To gain a better understanding of the cytotoxicity of aminoanthraquinone-derived agents, several structurally related doubly substituted compounds, bearing selected characteristic functional groups, were designed. The approach was to develop structure/activity relationships (SAR) for aminoanthraquinone derivatives with redox-active centers attached to the anthraquinone skeleton through spacer side chains at positions 1 and 5.

Results and Discussion. – The 1,5-disubstituted 9,10-aminoanthraquinones **1**–**19** were readily prepared by nucleophilic substitution of 1,5-dichloro-9,10-anthraquinone (**20**)[1]). Upon heating **20** with a large excess of a primary amine (RNH_2) in a glass mini-reactor in the presence of DMF for 30 min, the symmetrically disubstituted products **1**–**19** were obtained in 40–80% yield (see the *Table*). Prolonged heating favored side reactions, leading to tedious purification, but was required for the preparation of higher homologs (data not shown). The purities of the products were generally higher after

[1]) Compound **20** is a suitable starting material also for the synthesis of more-complex molecules because of the juxtaposition of the Cl-atoms and the carbonyl groups [27]. A key to developing such compounds lies in the stepwise nucleophilic substitution of the Cl-atoms. The characteristic structural feature of the derivatives is a potential redox-active center in the side chain rather than in the intercalative part of the molecule. Doubly bioreductive agents such as 'bis-nitroimidazoles' [28] or *N,N'*-dioxides [29] have previously demonstrated enhanced cytotoxicity and selectivity towards hypoxic tumor cells.

Table. *Chemical Yields of the Disubstituted 9,10-Anthraquinones* **1–19**, *and Their Cytotoxicities against Suspended Murine and Human Tumor Cell Lines*



20 **1 – 19**

Compound	R	Yield [%]	IC_{50} [μM][a])		
			Hep G2[b])	C6[c])	2.2.15[d])
1	Et	80	23.2 ± 1.1	94.4 ± 1.7	29.6 ± 1.0
2	$(CH_2)_2OH$	78	25.2 ± 1.1	49.37 ± 1.2	19.26 ± 2.2
3	i-Pr	63	7.64 ± 2.38	31.06 ± 1.5	18.98 ± 2.4
4	$(CH_2)_2NMe_2$	78	0.09 ± 0.01	0.12 ± 0.01	0.13 ± 0.01
5	$(CH_2)_2NH(CH_2)_2OH$	70	1.20 ± 0.02	1.17 ± 0.03	7.01 ± 0.11
6	Pr	73	1.74 ± 0.14	16.03 ± 0.68	1.94 ± 0.02
7	i-Bu	55	14.27 ± 1.54	12.67 ± 0.37	32.5 ± 1.1
8	$(CH_2)_3OH$	74	27.6 ± 1.1	94.7 ± 1.7	28.6 ± 0.9
9	$(CH_2)_3NH_2$	55	11.67 ± 0.09	12.18 ± 0.04	7.48 ± 0.09
10	Bu	72	> 200	> 200	> 200
11	$(CH_2)_4OH0$	65	133.0 ± 3.1	192.25 ± 4.1	15.5 ± 0.24
12	$(CH_2)_4NH_2$	65	11.61 ± 0.02	12.56 ± 0.16	10.07 ± 0.58
13	$(CH_2)_5NH_2$	40	17.37 ± 0.74	8.36 ± 0.13	18.7 ± 0.7
14	$(CH_2)_5Me$	65	> 200	> 200	> 200
15	Cyclopentyl	55	36.56 ± 1.1	98.76 ± 2.1	39.75 ± 1.1
16	cHex[e])	46	56.67 ± 2.1	134.6 ± 4.1	134.6 ± 3.5
17	4-(OH)-C_6H_4	56	4.20 ± 0.76	14.21 ± 0.08	32.5 ± 2.5
18	$CH_2C_6H_5$	75	185.5	130.9 ± 3.7	327 ± 5
19	$(CH_2)_2C_6H_5$	64	181.5 ± 7.5	217.1 ± 4.2	182.5 ± 5.1
Mitoxantrone			2.00 ± 0.5	0.07 ± 0.01	0.40 ± 0.02
Adriamycin			0.90 ± 0.01	1.00 ± 0.16	1.60 ± 0.04
Cisplatin			1.48 ± 0.62	> 1	2.0 ± 0.54

[a]) Drug concentration inhibiting 50% of cell growth after 48 h of exposure. The variance for IC_{50} was less than ±20%. Inhibition of cell growth was significantly different relative to that of the controls; $n \geq 3$, $P < 0.01$. [b]) Human hepatoma G2 cells. [c]) Rat glioma C6 cells. [d]) Hepatitis B virus transfected hepatoma cell lines. [e]) 2,3-Dimethylcyclohexyl.

crystallization. Concerning the choice of substituents, alkylamino groups were chosen such that the overall reduction potential of the anthraquinone nucleus moved to more-negative values [30]. The structures of **1–19** were corroborated spectroscopically (^{1}H-NMR, ^{13}C-NMR, MS).

The anthraquinone moiety is known to undergo redox processes that can give rise to cytotoxic effects [22][31][32]. We have initiated a structure-based approach towards discovering non-nucleosidic compounds that selectively inhibit tumor cell lines. Both the toxicity and cytotoxicity of 9,10-anthraquinone derivatives are greatly affected by substituents [33]. Mitoxantrone, an aminoanthraquinone first synthesized in 1979, displays antiviral, antibacterial, immunomodulatory, and antitumor activities. Its

antitumor activity is attributed to its interaction with DNA topoisomerase II, and its interaction with human cells may also involve nonintercalating, electrostatic interactions [33]. The current series of aminoanthraquinones were also designed as telomerase inhibitors (or activators) [16][34]. These include antisense compounds, reverse transcriptase inhibitors, and compounds capable of interacting with high-order telomeric-DNA-tetraplex ('G-quadruplex') structures, so as to prevent enzyme access to the necessary linear telomere substrate [35]. The cytotoxic *in vitro* activity of such drugs, as revealed by colorimetric assays, is mainly due to chromosome damage [36]. Aminoanthraquinones may represent a novel class of polyamine-binding-site ligands with a unique pharmacophore and may facilitate the rational design of novel NMDA (*N*-methyl-D-aspartate)-receptor modulators [37].

As shown in the *Table*, half of the compounds studied did show significant cytotoxicities ($IC_{50} < 20$ μM) towards tumor cells. Compound **4** showed substantial *in vitro* growth delays of Hep G2, 2.2.15, and C6 cells, the mechanism of action not being clear so far. Preliminary SAR studies on protein-kinase-C activity have suggested that the terminal Me_2N groups of **4** is crucial for PKC inhibition [25].

Compounds **4**–**6**, **9**, and **12** were tested *in vitro* against murine and human tumor cells, whose growth was inhibited at micromolar level (*Table*). When compound **4** was tested *in vitro* against Hep-G2 and hepatitis-B-virus-transfected hepatoma cell lines (2.2.15 cells), IC_{50} values of 0.09 and 0.13 μM were determined, respectively. Compounds **4**–**6** were very active, with IC_{50} values of 0.09, 1.20, and 1.74 μM, respectively, for the inhibition of cultured Hep-G2 cell growth, as determined by the XTT colorimetric assay (see *Exper. Part*), and their antiproliferative activity was markedly enhanced, comparable to that of mitoxantrone, adriamycin, and cisplatin, which had IC_{50} values of 2.00, 0.90 and 1.48 μM, respectively (positive controls). Indeed, compound **4** was more effective by a factor of *ca.* 22 than mitoxantrone, ten times more effective than adriamycin, and sixteen times more effective than cisplatin towards Hep-G2 cells. Thus, it appears that the anthraquinone chromophore itself causes the cytotoxicity, although the exact mechanism is still unclear. Further SAR studies of amino-substituted 9,10-anthraquinones are now underway to investigate the structural requirements of these interesting pharmacophores.

Experimental Part

General. All reactions were monitored by thin-layer chromatography (TLC; silica gel *60* F_{254}). Flash-column-chromatographic (FC) purifications were performed on silica gel (70–230 mesh; *Merck*) with CH_2Cl_2 as the eluent. Melting points (m.p.) were determined on a *Büchi B-545* apparatus and are uncorrected. FT-IR Spectra (KBr): *Perkin-Elmer 983G* spectrometer; in cm^{-1}. UV Spectra: *Shimadzu UV-160A*; λ_{max} in nm (log ε). ^{1}H-NMR: *Varian Gemini-300* (300 MHz) and *Brucker AM-500* (500 MHz); chemical shifts δ in ppm relative to $SiMe_4$ as internal standard, coupling constants J in Hz. Mass spectra (EI-MS; 70 eV, unless otherwise stated): *Finnigan MAT TSQ-46*, *Finnigan MAT TSQ-700* (Universität Regensburg, Germany), and *Finnigan MAT LCQ* (National Research Institute of Chinese Medicine, Taipei, Taiwan); in *m/z* (rel. %).

General Procedure for the Preparation of the 1,5-Disubstituted 9,10-Anthraquinones **1**–**19**. A mixture of 1,5-dichloro-9,10-anthraquinone (**20**; 1.0 g, 3.6 mmol) and *N,N*-dimethylformamide (DMF; 20 ml), containing the appropriate amine (8.0 mmol), was heated to 90° in a glass mini-reactor for 30 min under N_2 gas. After cooling, the mixture was treated with crushed ice. The resulting precipitate was collected by filtration, washed with plenty of H_2O, and purified by recrystallization from AcOEt/hexane to afford the final products as red needles.

1,5-Bis(ethylamino)-9,10-anthraquinone (**1**). Yield: 80%. M.p. 193–195° (AcOEt/hexane). UV (MeOH): 254 (4.50), 514 (1.49). IR (KBr): 3289, 2929, 1649. ^{1}H-NMR ($CDCl_3$): 1.41–1.47 (*m*, 6 H); 3.39 (*q*, *J* = 5.2, 4 H); 6.98 (*d*, *J* = 7.5, 2 H); 7.53–7.58 (*m*, 2 H); 8.31 (*d*, *J* = 3.0, 2 H); 9.65 (br., 2 H). ^{13}C-NMR ($CDCl_3$): 14.4; 37.4; 115.7; 116.2; 126.2; 133.7; 135.0; 151.3; 185.4. EI-MS: 294 (M^+), 264.

1,5-Bis[(2-hydroxyethyl)amino]-9,10-anthraquinone (**2**). Yield: 78%. M.p. 229–230° (AcOEt/hexane). UV (MeOH): 521 (0.69), 282 (0.63). IR (KBr): 3370, 1646. ^{1}H-NMR ((D_6)DMSO): 3.63 (*d*, *J* = 2.4, 4 H); 4.06 (*s*, 2 H); 5.35 (*d*, *J* = 3.6, 4 H); 7.53 (*d*, *J* = 8.4, 2 H); 7.79 (*d*, *J* = 7.2, 2 H); 7.96 (*t*, *J* = 8.1, 2 H); 10.16 (br., 2 H). ^{13}C-NMR ((D_6)DMSO): 49.99; 64.59; 117.26; 119.40; 122.32; 140.65; 140.74; 156.39; 185.36. EI-MS: 326 (M^+), 295, 264.

1,5-Bis(isopropylamino)-9,10-anthraquinone (**3**). Yield: 65%. M.p. 170–172° (AcOEt/hexane). UV (MeOH): 527 (0.33), 284 (0.26). IR (KBr): 3289, 1648. ^{1}H-NMR ($CDCl_3$): 1.38 (*d*, *J* = 6.0, 12 H); 3.85 (*q*, *J* = 6.6, 2 H); 7.01 (*d*, *J* = 3.3, 2 H); 7.51 (*d*, *J* = 7.5, 2 H); 7.55 (*t*, *J* = 3.7, 2 H); 9.79 (*d*, *J* = 6.0, 2 H). ^{13}C-NMR ($CDCl_3$): 22.45; 43.30; 112.48; 114.05; 116.25; 134.68; 136.24; 150.29; 185.00. EI-MS: 322 (M^+), 312, 264.

1,5-Bis[[2-(dimethylamino)ethyl]amino]-9,10-anthraquinone (**4**). Yield: 78%. M.p. 187–188° (AcOEt/hexane; lit. [38]: 188–190°). UV (MeOH): 522 (0.42), 282 (0.38). IR (KBr): 3277, 1642. ^{1}H-NMR ($CDCl_3$): 2.39 (*s*, 12 H); 2.69 (*t*, *J* = 6.4, 4 H); 3.46 (*q*, *J* = 6.0, 4 H); 6.99 (*d*, *J* = 8.4, 2 H); 7.52–7.63 (*m*, 4 H); 9.80 (br., 2 H). ^{13}C-NMR ((D_6)DMSO): 41.02; 45.52; 58.12; 113.21; 114.87; 116.20; 135.05; 136.34; 151.18; 185.35. EI-MS: 380 (M^+), 335, 264.

1,5-Bis{[2-[(2-hydroxyethyl)amino]ethyl]amino}-9,10-anthraquinone (**5**). Yield: 70%. M.p. 185–186° (AcOEt/hexane; lit. [38]: 185°). UV (MeOH): 522 (1.07), 281 (1.15). IR (KBr): 3400, 3288, 1640, 1590. ^{1}H-NMR ((D_6)DMSO): 2.66 (*d*, *J* = 5.7, 4 H); 3.34 (*t*, *J* = 10.8, 8 H); 3.50 (*s*, 2 H); 4.54 (*s*, 2 H); 7.18 (*d*, *J* = 6.0, 2 H); 7.41 (*t*, *J* = 7.0, 2 H); 7.61 (*t*, *J* = 7.0, 2 H); 9.79 (*s*, 2 H). ^{13}C-NMR ((D_6)DMSO): 42.45; 48.00; 51.48; 60.54; 112.15; 114.31; 117.23; 135.60; 135.70; 151.18; 184.26. EI-MS: 412 (M^+), 351, 339, 278 (100), 265, 252.

1,5-Bis(propylamino)-9,10-anthraquinone (**6**). Yield: 73%. M.p. 153–154° (AcOEt/hexane). UV (MeOH): 524 (0.16), 283 (0.13). IR (KBr): 3330, 1640. ^{1}H-NMR ($CDCl_3$): 1.12 (*t*, *J* = 7.5, 6 H); 1.81 (*q*, *J* = 7.1, 4 H); 3.38 (*q*, *J* = 6.4, 4 H); 7.01 (*d*, *J* = 7.8, 2 H); 7.54 (*t*, *J* = 7.3, 2 H); 7.60 (*t*, *J* = 2.4, 2 H); 9.79 (br., 2 H). ^{13}C-NMR ($CDCl_3$): 11.70; 22.27; 45.27; 113.33; 115.43; 117.22; 135.21; 136.20; 150.82; 185.34. EI-MS: 322 (M^+), 293 (100), 264, 236.

1,5-Bis(isobutylamino)-9,10-anthraquinone (**7**). Yield: 55%. M.p. 167–168° (AcOEt/hexane). UV (MeOH): 527 (0.35), 284 (0.26). IR (KBr): 3359, 1624. ^{1}H-NMR ($CDCl_3$): 1.11 (*d*, *J* = 6.3, 12 H); 2.00–2.10 (*m*, 2 H); 3.20 (*q*, *J* = 6.3, 4 H); 6.98 (*d*, *J* = 7.8, 2 H); 7.51 (*d*, *J* = 7.8, 2 H); 7.56–7.60 (*m*, 2 H); 9.88 (br., 2 H). ^{13}C-NMR ($CDCl_3$): 20.56; 28.08; 50.74; 112.85; 114.56; 116.35; 135.08; 136.33; 151.66; 185.42. APCI-MS: 351 (M^+), 352 (24), 295, 279, 254.

1,5-Bis[(3-hydroxypropyl)amino]-9,10-anthraquinone (**8**). Yield: 74%. M.p. 178–180° (AcOEt/hexane). UV (MeOH): 522 (0.41), 283 (0.41). IR (KBr): 3359, 1624. ^{1}H-NMR ((D_6)DMSO): 1.74–1.83 (*m*, 4 H); 3.37 (*t*, *J* = 6.6, 4 H); 3.51 (*q*, *J* = 5.6, 4 H); 4.63 (*t*, *J* = 4.6, 2 H); 7.12 (*d*, *J* = 8.7, 2 H); 7.38 (*d*, *J* = 7.5, 2 H); 7.57 (*t*, *J* = 7.9, 2 H); 9.65 (*t*, *J* = 5.2, 2 H). ^{13}C-NMR ((D_6)DMSO): 31.96; 38.35; 58.41; 112.08; 114.33; 117.10; 135.55; 135.66; 151.27; 184.37. APCI-MS: 355 (M^+), 356 (25).

1,5-Bis[(3-aminopropyl)amino]-9,10-anthraquinone (**9**). Yield: 55%. M.p. 153–155° (AcOEt/hexane). UV (MeOH): 514 (1.95), 281 (1.52). IR (KBr): 3330, 1640. ^{1}H-NMR ($CDCl_3$): 1.92 (*t*, *J* = 4.8, 4 H); 1.97 (*q*, *J* = 4.5, 4 H); 2.96 (*q*, *J* = 4.5, 4 H); 3.44 (*q*, *J* = 6.9, 4 H); 7.04 (*t*, *J* = 3.7, 2 H); 7.52–7.62 (*m*, 4 H); 9.79 (*s*, 2 H). ^{13}C-NMR ($CDCl_3$): 32.93; 39.84; 40.52; 114.74; 115.88; 116.29; 135.12; 136.33; 151.47; 185.44. APCI-MS: 323 (100, $[M+1]^+$), 322 (M^+).

1,5-Bis(butylamino)-9,10-anthraquinone (**10**). Yield: 72%. M.p. 152–153° (AcOEt/hexane). UV (MeOH): 527 (0.25), 283 (0.19). IR (KBr): 3299, 2947, 1623, 1500. ^{1}H-NMR ($CDCl_3$): 1.02 (*q*, *J* = 4.9, 6 H); 1.50–1.60 (*m*, 4 H); 1.74–1.84 (*m*, 4 H); 3.33 (*q*, *J* = 6.4, 4 H); 7.00 (*d*, *J* = 7.5, 2 H); 7.52 (*t*, *J* = 9.0, 2 H); 7.57 (*q*, *J* = 3.0, 2 H); 9.79 (br., 2 H). ^{13}C-NMR ($CDCl_3$): 13.75; 20.32; 31.22; 42.62; 112.92; 114.55; 116.25; 135.05; 136.38; 151.54; 185.41. APCI-MS: 352 (M^+), 351 (100).

1,5-Bis[(4-hydroxybutyl)amino]-9,10-anthraquinone (**11**). Yield: 65%. M.p. 120–122° (AcOEt/hexane). UV (MeOH): 523 (1.30), 284 (1.10). IR (KBr): 3384, 1643. ^{1}H-NMR ((D_6)DMSO): 1.78 (*q*, *J* = 4.6, 4 H); 1.86 (*q*, *J* = 4.3, 4 H); 3.39 (*t*, *J* = 6.6, 4 H); 3.76 (*t*, *J* = 6.1, 4 H); 4.18 (*s*, 2 H); 7.00 (*d*, *J* = 6.3, 2 H); 7.52–7.61 (*m*, 4 H); 9.77 (br., 2 H). ^{13}C-NMR ((D_6)DMSO): 25.50; 30.10; 60.58; 63.60; 112.08; 114.38; 117.20; 135.55; 135.69; 151.26; 184.42. EI-MS: 382 (M^+), 365, 291, 253 (100).

1,5-Bis[(4-aminobutyl)amino]-9,10-anthraquinone (**12**). Yield: 65%. M.p. 208–210° (AcOEt/hexane). UV (MeOH): 514 (1.17), 280 (1.02). IR (KBr): 3282, 1641, 1594. ^{1}H-NMR ($CDCl_3$): 1.66–1.73 (*q*, *J* = 10.6, 4 H);

1.82 (*q*, *J* = 6.3, 4 H); 2.73–2.83 (*m*, 4 H); 2.84 (*t*, *J* = 3.4, 4 H); 3.36 (*t*, *J* = 6.3, 4 H); 6.99 (*d*, *J* = 7.5, 2 H); 7.52–7.60 (*m*, 4 H); 9.76 (*s*, 2 H). APCI-MS: 381 (M^+), 364, 309.

1,5-Bis[(5-aminopentyl)amino]-9,10-anthraquinone (**13**). Yield: 40%. M.p. 113–115° (AcOEt/hexane). UV (MeOH): 524 (0.47), 283 (0.45). IR (KBr): 3285, 1646. ^{1}H-NMR ($CDCl_3$): 0.87 (*t*, *J* = 7.6, 4 H); 1.56 (*d*, *J* = 3.3, 8 H); 1.80 (*d*, *J* = 7.5, 8 H); 2.75 (*t*, *J* = 6.6, 4 H); 3.34 (*q*, *J* = 6.3, 4 H); 6.98 (*q*, *J* = 3.1, 2 H); 7.52–7.59 (*m*, 4 H); 9.75 (*s*, 2 H). APCI-MS: 410 (20, $[M+1]^+$), 409 (M^+).

1,5-Bis(hexylamino)-9,10-anthraquinone (**14**). Yield: 65%. M.p. 142–144° (AcOEt/hexane). UV (MeOH): 518 (0.15). IR (KBr): 3388, 1626. ^{1}H-NMR ($CDCl_3$): 0.95 (*t*, *J* = 3.5, 6 H); 1.37 (*q*, *J* = 3.5, 4 H); 1.47 (*t*, *J* = 7.2, 4 H); 1.75 (*t*, *J* = 7.3, 4 H); 1.82 (*d*, *J* = 6.9, 4 H); 3.32 (*t*, *J* = 5.4, 4 H); 6.99 (*d*, *J* = 6.0, 2 H); 7.52 (*t*, *J* = 7.5, 2 H); 7.58 (*q*, *J* = 2.2, 2 H); 9.74 (br., 2 H). ^{13}C-NMR ($CDCl_3$): 13.92; 22.50; 26.75; 29.29; 31.52; 42.97; 114.53; 116.23; 127.86; 135.03; 136.38; 151.51; 185.39. APCI-MS: 407 (100, $[M+1]^+$), 406 (M^+).

1,5-Bis(cyclopentylamino)-9,10-anthraquinone (**15**). Yield: 55%. M.p. 198–200° (AcOEt/hexane). UV (MeOH): 528 (0.39), 285 (0.30). IR (KBr): 3388, 1640. ^{1}H-NMR ($CDCl_3$): 1.72–1.78 (*m*, 8 H); 1.84 (*t*, *J* = 5.7, 4 H); 2.11 (*t*, *J* = 5.8, 4 H); 4.04 (*d*, *J* = 4.8, 2 H); 7.03 (*q*, *J* = 3.2, 2 H); 7.51 (*d*, *J* = 7.5, 2 H); 7.56 (*t*, *J* = 3.7, 2 H); 9.86 (*d*, *J* = 5.4, 2 H). ^{13}C-NMR ($CDCl_3$): 24.08; 33.58; 53.88; 112.92; 114.46; 117.10; 134.89; 136.44; 151.0; 185.29. FAB-MS: 375 (M^+), 307 (28).

1,5-Bis[(2,3-dimethylcyclohex-1-yl)amino]-9,10-anthraquinone (**16**). Yield: 46%. M.p. 188–190° (AcOEt/hexane). UV (MeOH): 527 (0.47), 281 (0.36). IR (KBr): 3273, 1676. ^{1}H-NMR ($CDCl_3$): 2.00 (*s*, 12 H); 2.14–3.67 (*m*, 16 H); 3.90 (*s*, 2 H); 7.00–9.99 (*m*, 6 H); 10.20 (br., 2 H). ^{13}C-NMR ($CDCl_3$): 17.05; 20.72; 25.09; 33.61; 35.11; 38.57; 45.44; 57.46; 114.28; 116.50; 116.83; 134.97; 136.67; 151.57; 185.31. APCI-MS: 459 (M^+), 460 (25).

1,5-Bis[(4-hydroxyphenyl)amino]-9,10-anthraquinone (**17**). Yield: 56%. M.p. 211–212° (AcOEt/hexane). IR (KBr): 3420, 3323, 1642. UV (MeOH): 517 (0.28), 252 (1.49). ^{1}H-NMR ($CDCl_3$): 4.90 (*s*, 4 H); 6.92 (*d*, *J* = 8.7, 2 H); 7.21 (*t*, 4 H); 7.48 (*t*, *J* = 8.1, 2 H); 7.65–7.84 (*m*, 2 H); 8.30–8.38 (*m*, 2 H); 11.07 (*s*, 2 H). APCI-MS: 423 (M^+), 352 (35).

1,5-Bis[(phenylmethyl)amino]-9,10-anthraquinone (**18**). Yield: 75%. M.p. 218–220° (AcOEt/hexane). UV (MeOH): 518 (0.59), 281 (0.71). IR (KBr): 3270, 1640. ^{1}H-NMR ($CDCl_3$): 4.61 (*d*, *J* = 6.0, 4 H); 6.95 (*d*, *J* = 8.4, 2 H); 7.31–7.40 (*m*, 6 H); 7.41 (*t*, *J* = 3.9, 2 H); 7.63 (*d*, *J* = 3.0, 2 H); 10.15 (br., 2 H). ^{13}C-NMR ($CDCl_3$): 47.0; 115.27; 116.84; 127.0; 127.31; 128.74; 135.12; 136.24; 138.12; 151.21; 185.38. EI-MS: 418 (M^+), 347 (60).

1,5-Bis[(phenylethyl)amino]-9,10-anthraquinone (**19**). Yield: 64%. M.p. 205–207° (AcOEt/hexane). UV (MeOH): 527 (0.13), 285 (0.28). IR (KBr): 3270, 1640. ^{1}H-NMR ($CDCl_3$): 3.07 (*t*, *J* = 7.5, 2 H); 3.58–3.65 (*m*, *J* = 5.1, 2 H); 7.00 (*d*, *J* = 8.1, 2 H); 7.31 (*t*, *J* = 6.0, 2 H); 7.36–7.41 (*m*, 4 H); 7.55 (*t*, *J* = 5.3, 2 H); 7.59 (d, *J* = 5.7, 2 H); 9.83 (br., 2 H). ^{13}C-NMR ($CDCl_3$): 35.71; 44.61; 113.13; 114.88; 116.20; 126.52; 128.69; 135.07; 136.32; 138.87; 151.15; 185.36. APCI-MS: 448 (35, $[M+1]^+$), 447 (M^+).

Cell Cultures. Various cancer-cell lines (G2, 2.2.15, and C6 cells) were cultured in minimum essential medium (MEM), supplemented with 10% fetal calf serum, penicillin (100 U/ml), and streptomycin (100 mg/ml) in a humidified atmosphere with 5% CO_2 at 37°. Cell-culture media were renewed every 3 d, up to the confluence of the monolayer. When the cells had formed confluent cultures, they were detached from the culture flasks or dishes by means of the trypsin/EDTA ('ethylenediamine tetraacetate') reagent. Test compounds were stored at −70° before dissolved in neat DMSO. All drug solns. were prepared immediately before evaluation, and were transfered into complete medium before addition to the cell cultures. The 'inhibitory concentration fifty' (IC_{50}) values (see the *Table*) were determined after 48 h of drug exposure and represent the mean values of independent measurements performed at least in triplicate, showing the same pattern of expression.

XTT Color Assay. The reagent XTT (=2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2*H*-tetrazolium hydroxide) forms a suitably colored, water-soluble, nontoxic formazan upon metabolic reduction by viable cells. A total of *ca.* 2×10^3 cells, suspended in MEM, were plated onto each well of a 96-well plate, and incubated in 5% CO_2 at 37° for 24 h. Test compounds were then added to the culture medium for each designated concentration. After 72 h, fresh XTT (50 µl) and the electron-coupling reagent PMS (='*N*-methyl dibenzopyrazin methyl sulfate'; 1 µl) were mixed together and then added to each well. After incubation at 37° for 6 h, the absorbance at 490 nm was measured with an ELISA reader.

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